PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of	Group Art Unit: 1804
	Examiner: Moody
JON DONSON, et al.	DECLARATION BY DR. LAURENCE K. GRILL UNDER 37 CFR §1.132
Serial No. 07/739,143	
Filed: August 1, 1991	LIMBACH & LIMBACH 2001 Ferry Building San Francisco, CA 94111 415/433-4150
For: RECOMBINANT PLANT VIRAL NUCLEIC ACIDS	
	Docket No. BIOG-20120 USA

Honorable Commissioner of Patents
and Trademarks
Washington, D.C. 20231

Sir:

I, Dr. Laurence K. Grill, declare and state as follows:

1. I am employed by Biosource Genetics Corporation as Vice President of Research and Development. I have worked at Biosource Genetics Corporation since 1987. Attached hereto is my curriculum vitae.

2. I am a co-inventor of the above-referenced application and as such am familiar with the specification and claims of the above-identified patent application and the Office Action mailed June 15, 1993 (Paper No. 16).

3. The present invention relates to a recombinant viral nucleic acid possessing enhanced

stability within a host, thereby enabling the sustained systemic transcription of a nucleic acid sequence within the host. Enhanced stability within the host has been accomplished using a dual subgenomic promoter system which is believed to reduce the frequency of recombination leading to the regeneration of the wild type virus.

The present invention may employ any recombinant viral nucleic acid derived from a plus sense, single stranded RNA virus that naturally has a subgenomic promoter. A number of single-stranded RNA viruses have smaller, subgenomic mRNAs that are synthesized in the host's cells after infection. The transcription of these subgenomic RNAs depend on sequences that are upstream (5') and are referred to as internal or subgenomic promoters. A good description of subgenomic promoters is presented in R. E. F. Matthews, Plant Virology, 3rd Edition, Academic Press, Inc., San Diego p.180 (1991) which is attached hereto as Exhibit 1.

Plus sense, single stranded RNA plant viruses that naturally possess a subgenomic promoter include tobamoviruses, bromoviruses, tobraviruses, furoviruses, cucumoviruses, hordeiviruses, potexviruses, tymoviruses, luteoviruses, carmoviruses, tombusviruses and sobemoviruses and animal viruses. Plus sense, single stranded RNA animal viruses that naturally possess a subgenomic promoter include alphaviruses such as the sinbis virus.

Any subgenomic promoter from a plus sense, single stranded RNA virus that naturally possesses a subgenomic promoter may be used in the present invention. Subgenomic promoters from a variety of plus-sense RNA viruses have been employed. Table 1,

attached hereto as Exhibit 2, describes a series of functional vectors that have been prepared according to the present invention. Subgenomic promoters used in the vectors described in Table 1 include tobacco mosaic virus (TMV) and odontoglossum ringspot virus (ORSV).

As demonstrated by the functional vectors depicted in Table 1, neither the subgenomic promoter that regulates transcription of the coat protein nor the subgenomic promoter that regulates transcription of the second nucleic acid sequence needs to be native to the recombinant viral nucleic acid. Given that transcription of the coat protein and the second nucleic acid sequence are not interdependent, there is no requirement that either the subgenomic promoter for the coat protein sequence or the second nucleic acid sequence be native to the vector.

The only requirement placed on the subgenomic promoters used in the dual promoter system of the present invention is that the two subgenomic promoters possess nucleic acid sequences that are sufficiently different from each other so as to enable the recombinant viral nucleic acid to systemically transcribe the second nucleic acid in the host. In order to enable systemic transcription in the host, the two subgenomic promoters need to have different nucleic acid sequences to reduce the frequency of recombination leading to the generation of the wild type strain. By reducing the rate at which the wild type strain is regenerated, the recombinant viral nucleic acid is stabilized, enabling systemic infection within the host.

The dual promoter system of the present invention has been shown to be significantly more stable than prior art promoter systems. Two such prior art

systems are taught by Ahlquist, et al. which teaches that the foreign gene may be expressed either as a fusion protein or by using the same subgenomic promoter as is used to express the coat protein. See Ahlquist, et al., Col. 9, lines 48-58.

Donson, et al. teaches that

> vector constructs have also been constructed with an additional viral subgenomic promoter to express a foreign gene. However, on infection of plants, these vectors had the added sequences deleted and failed to be transported systematically. This was hypothesized to be from recombination between the two repeated subgenomic promoter sequences within the viral constructs.

Donson, et al., page 7204, column 2. The key difference between the prior art vectors described by Ahlquist, et al. and Donson, et al. and the vectors of the present invention is the fact that the subgenomic promoter sequences for the coat protein sequence and the second nucleic acid sequence are not repeated but rather are different. The functional significance of this distinction is demonstrated by the examples set forth in the Specification.

The comparative examples presented in the Specification clearly teach that vectors where the second nucleotide sequence is transcribed as a fusion protein (Comparative Example 1, Specification, page 37) or where identical subgenomic promoters are used (Comparative Example 2, Specification, page 44) are not capable of sustained systemic infection. Specifically, the Specification teaches that when the second nucleotide sequence is transcribed as a fusion protein,

> the amounts of CAT activity in upper, systemically infected leaves were variable and much lower than in inoculated leaves, and in many cases none was detected. Hybridizations with TMV and CAT probes demonstrated that the proportion

of virus-retaining CAT sequences was quickly reduced to undetectable levels.

Specification, page 41, lines 28-34. The Specification teaches that the loss of CAT sequence is possibly due to homologous recombination as has been described in other plus-sense RNA viruses. See Kirkegaard, K. and Baltimore, D., Cell 47:433 (1986); Bujarski, J. and Kaesberg, P., Nature 321:528 (1986); King, A.M.Q., in RNA Genetics, E. Domingo, et al., Eds., Vol. II, 149-165, CRC Press, Inc., Boca Raton, Fla. (1988).

The Specification also teaches that a vector employing identical subgenomic promoters for both the coat protein sequence and the second nucleic acid sequence is not capable of sustained systemic infection. See Specification, page 44, line 1 - page 45, line 7. Transmissibility of this vector was found to be one hundredth that of the CAT-coat protein fusion vector and wild-type TMV. No systemic infection of even the wild-type vector was observed when the same promoters are employed. This indicates that the hybrid virus does not delete the inserted sequences in a manner to create a wild-type-like virus. Specification, page 45, lines 1-7.

By contrast to comparative examples 1 and 2, the present Specification teaches that vectors which employ two different subgenomic promoters that do not recombine are able to systemically infect the host without loss of transcription of the nucleic acid sequence over a 48 day period. Specification, page 52, lines 7-11. Such sustained systemic infection and transcription is made possible by the use of subgenomic promoters that are sufficiently different that they do not readily recombine.

The results presented in Kumagai, et al., Proc. Natl. Acad. Sci. (1993) 90:427-430, attached hereto

as Exhibit 3, evidence the present invention's improvement over the prior art. Using a vector designed according to the present invention where the second nucleic acid sequence encodes for α-trichosanthin, Kumagai, et al. systemically infected N. benthamiana and expressed α-trichosanthin at levels of at least 2% of the total soluble protein. 2% of the soluble protein is the highest accumulation of a foreign protein ever reported in any genetically engineered plant.

4. I further declare, under penalty of perjury under the laws of the United States of America, that all statements made herein of my own knowledge are true and that these statements were made with the knowledge that willful false statements and the like are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code.

Date: 11/8/93

Dr. Laurence K. Grill

ATTY DOCKET: BIOG 20120

Curriculum Vitae

Laurence K. Grill
Biosource Genetics Corporation
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EDUCATION:

Ph.D. Plant Pathology, University of California at Riverside
Emphasis: plant molecular biology and virology August 1979

M.A. Biological Sciences, California State University at Fullerton
Inst. of Molecular Biology, Emphasis: virology January 1976

B.A. Biological Sciences, California State University at Fullerton
Emphasis: genetics June 1973

PROFESSIONAL EXPERIENCE:

Vice President of Research - Oct. 1987 to present, BIOSOURCE GENETICS CORPORATION

Molecular Biology research Manager - June 1984 to July 1987, ZOECON RESEARCH INSTITUTE, SANDOZ CROP PROTECTION CORP.

Senior Research Scientist - January 1980 to June 1984, ZOECON RESEARCH INSTITUTE

Post-doctoral Researcher - September 1979 to December 1979, Department of Plant Pathology, University of California, Riverside

Research Assistant - October 1975 to September 1979, Department of Plant Pathology, University of California, Riverside

Teaching Assistant - January 1974 to August 1975, Department of Biological Sciences, California State University, Fullerton

PROFESSIONAL ASSOCIATIONS:

Editor: Plant Molecular Biology Reporter, 1982 to present
Plant Cell Reports, 1985 to present

Member: International Society of Plant Molecular Biology
American Society of Virology
American Phytopathology Society

RELEVANT PUBLICATIONS:

Turpen, T., Garger, S.J., and Grill, L.K. 1988. *On the mechanism of cytoplasmic male sterility in the 447 line of* Vicia faba. **Plant Molecular Biology** 10:489-497.

Turpen, T., Garger, S.J., Marks, M.D. and Grill, L.K. 1987. *Molecular cloning and physical characterization of a* Brassica *linear mitochondrial plasmid.* **Mol. Gen. Genet.** 209:227-233.

Grill, L.K., Garger, S.J., Turpen, T.H., Lommel, S.A., Marsden, M.P.F. and Murry, L.E. 1983. *Involvement of viruses and virus-like agents with the male sterility traits in plants..* IN: **UCLA Symposia on Molecular and Cellular Biology, Vol. 12, Plant Molecular Biology,** Alan R. Liss, Inc., pp 101-116.

Grill, L.K 1983. *Utilizing RNA viruses for plant improvement.* **Pl. Mol. Biol. Rep.** 1:17-20.

Garger, S.J., Turpen, T.H., Carrington, J.C., Morris, T.J., Jordan, R.L., Dodds, J.A. and Grill, L.K. 1983. *Rapid detection of plant RNA viruses by dot blot hybridization.* **Pl. Mol. Biol. Rep.** 1:21-25.

Grill, L.K. and Garger, S.J. 1981 *Identification and characterization of double-stranded RNA associated with cytoplasmic male sterility.* **Proc. Natl. Acad. Sci., U.S.A.** 78:7043-7046.

Garger, S.J., Griffith, O.M. and Grill, L.K. 1983. *Rapid purification of plasmid DNA by a single centrifugation in a two-step cesium chloride-ethidium bromide gradient* . **Biochem. Biophys. Res. Comm.** 117:835-842

Grill, L.K. and Semancik, J.S. 1980 *Properties of the complementary RNA sequences associated with infection by the citrus exocortis viroid..* **Virology** 107:24-33.

Negruk, V.I., Grill, L.K. and Semancik, J.S. 1980 *In vitro 32P labelling of viroid. RNA for hybridization studies..* **J. Virological Methods** 1:229-234.

Grill, L.K. and Semancik, J.S. 1980 *Viroid. synthesis: A question of inhibition by actinomycin D..* **Nature** 283:399-400.

Grill, L.K. and Semancik, J.S. 1978 *RNA sequences complementary to citrus exocortis viroid. in nucleic acid preparations from infected* Gynura aurantiaca. **Proc. Natl. Acad. Sci., U.S.A.** 75:896-900.

#34

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of	Group Art Unit: 1804
	Examiner: Moody
JON DONSON, et al.	DECLARATION BY DR. WILLIAM O. DAWSON UNDER 37 CFR § 1.132
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	Docket No. BIOG-20120 USA

Honorable Commissioner of Patents
and Trademarks
Washington, D.C. 20231

Sir:

I, Dr. William O. Dawson declare and state as follows:

1. I am a Professor at the University of Florida. I am also a co-inventor of the above-referenced application and a co-author of Donson, et al. Proc. Natl. Acad. Sci. USA (1991) 88:7204-7208. I am also a consultant for Biosource Genetics Corporation. Attached hereto is my curriculum vitae.

2. I am familiar with the Specification and claims of the above-referenced patent application as well as Donson, et al. Proc. Natl. Acad. Sci. USA (1991) 88:7204-7208. I am also familiar with the Office Action mailed June 15, 1993 in the above-referenced application.

3. The Examiner has rejected the present application under 35 U.S.C. § 102(f) because not all of the authors of Donson, et al. were named as co-inventors of the above-referenced application, specifically Christopher M. Kearney and Mark E. Hilf.

At the time the research described in Donson, et al. was performed, Christopher M. Kearney was a post-doctorate student under my supervision at the University of California, Riverside. Christopher M. Kearney's contribution to the Donson, et al. article consists of characterizing some of the vectors described in the paper after they had been prepared. As such, Christopher M. Kearney did not contributed to the conception or reduction to practice of the present invention. Therefore, Christopher M. Kearney is not an inventor of the above-referenced application.

Mark E. Hilf was a graduate student working under my supervision at the time the research described in Donson, et al. was performed. Mark E. Hilf sequenced portions of the odontoglossum ringspot virus and prepared DNA fragments containing the subgenomic promoter and coat protein that were ultimately used to prepare some of the vectors described in the present invention. However, Mark E. Hilf did not sequence odontoglossum ringspot virus or prepare the DNA fragments with the present invention in mind and was not involved with the conception and reduction to practice of the above-referenced application. Therefore, Mark E. Hilf is not an inventor of the above-referenced application.

4. Jon Donson, George L. Grantham, Thomas H. Turpen, Ann M. Turpen, Stephen J. Garger, Laurence K. Grill and myself are named as co-inventors of the

above-referenced application. Each named co-inventor contributed to the conception and reduction to practice of at least one of the pending claims and therefore should be considered a co-inventor of the above-referenced application.

5. I further declare, under penalty of perjury under the laws of the United States of America, that all statements made herein of my own knowledge are true and that these statements were made with the knowledge that willful false statements and the like are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code.

Date: 11/1/93

Dr. William O. Dawson

Curriculum Vitae

WILLIAM O. DAWSON

Title: Professor

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Education: BS - University of Georgia - 1966
PhD in Plant Pathology - University of Georgia - 1971

Research and Professional Experience:

1992 - present	Eminent Professor, University of Florida, Lake Alfred
1984 - 1992	Professor, University of California, Riverside
1978 - 1984	Assoc. Prof., University of California, Riverside
1972 - 1978	Assist. Prof., University of California, Riverside
1971 - 1972	Postdoctoral, University of California, Berkeley

Refereed Publications, last 5 years:

Culver, J. N., and W. O. Dawson. 1989. Point mutations in the coat protein gene of tobacco mosaic virus induce hypersensitivity in Nicotiana sylvestris. Molec. Plant-Microbe Interactions 2: 209-213.

Aldaoud, R., W. O. Dawson, and G. E. Jones. 1989. Rapid, random evolution of the genetic structure of replicating tobacco mosaic virus populations. Intervirology 30: 227-233.

Dawson, W. O., D. L. Lewandowski, M. E. Hilf, P. Bubrick, A. J. Raffo, J. J. Shaw, G. L. Grantham, and P. R. Desjardins. 1989. A tobacco mosaic virus-hybrid expresses and loses an added gene. Virology 172: 285-292.

Culver, J. N., and W. O. Dawson. 1989. Tobacco mosaic virus coat protein: an elicitor of the hypersensitive reaction but not required for the development of mosaic symptoms in Nicotiana sylvestris. Virology 173: 755-758.

Lehto, K., G. L. Grantham, and W. O. Dawson. 1990. Insertion of sequences containing the coat protein subgenomic RNA promoter and leader in front of the tobacco mosaic virus 30K ORF delays its expression and causes defective cell-to-cell movement. Virology 174: 145-157.

Lehto, K., and W. O. Dawson. 1990. Changing the start codon of the 30K gene of tobacco mosaic virus from "weak" to "strong" does not increase expression. Virology 174: 169-176.

Lehto, K., P. Bubrick, and W. O. Dawson. 1990. Time course of TMV 30K protein accumulation in intact leaves. Virology 174: 290-293.

EXHIBIT
1

Lehto, K., and W. O. Dawson. 1990. Replication, stability, and gene expression of tobacco mosaic virus mutants with a second 30K ORF. Virology 175: 30-40.

Beck, D. L., and W. O. Dawson. 1990. Deletion of repeated sequences from tobacco mosaic virus mutants with two coat protein genes. Virology 177: 462-469.

Iltis, R., J. G. Bald, S. M. Schneider, and W. O. Dawson. 1989. Logistic and poisson models for infection by multicomponent plant viruses. J. Virol. Meth. 26: 147-158.

Raffo, A. J., and Dawson, W. O. 1991. Construction of tobacco mosaic virus subgenomic replicons that are replicated and spread systemically in tobacco plants. Virology 184:277-289.

Donson, J., Kearney, C. M., Hilf, M. E., and Dawson, W. O. 1991. Systemic expression of a bacterial gene by a tobacco mosaic virus-based vector. Proc. Natl. Acad. Sci. USA. 88:7204-7208.

Lindbeck, A. G. C., Dawson, W. O., and Thomson, W. W. 1991. Coat protein-related polypeptides from in vitro tobacco mosaic virus coat protein mutants do not accumulate in the chloroplasts of directly inoculated leaves. Molec. Plant-Microbe Interactions 4:89-94.

Culver, J. N., and Dawson, W. O. 1991. Tobacco mosaic virus elicitor coat protein genes produce a hypersensitive phenotype in transgenic Nicotiana sylvestris plants. Molec. Plant-Microbe Interactions 4:458-463.

Dawson, W. O. 1992. Tobamovirus-plant interactions. (Minireview). Virology 186: 359-367.

Hilf, M. E., and Dawson, W. O. 1993. The tobamovirus capsid protein functions as a host range determinant of long-distance movement. Virology 186:359-367.

Lindbeck, A. G. C., Lewandowski, D. J., Culver, J. N., Thomson, W. W., and Dawson, W. O. 1992. Mutant coat protein of tobacco mosaic virus induces acute chlorosis in expanded and developing tobacco leaves. Molec. Plant-Microbe Interact. 5:235-241.

Kearney, C. M., Donson, J., Jones, G. E., and Dawson, W. O. 1993. Low level of genetic drift in foreign sequences replicating in an RNA virus in plants. Virology 192:11-17.

Culver, J. N., Lehto, K., Close, S. M., Hilf, M. E., and Dawson, W. O. 1993. Genetic position affects the expression of tobacco mosaic virus movement and coat protein genes. Proc. Natl. Acad. Sci. USA 90:2055-2059.

Lewandowski, D. J., and Dawson, W. O. 1993. A single amino acid change in tobacco mosaic virus replicase prevents symptom production. Molec. Plant-Microbe Interact. 6:157-160.

Kumagai, M., Turpen, T., Weinzettl, N., dellaCioppa, G., Turpen, A., Donson, J., Hilf, M., Grantham, G., Dawson, W., Chow, T., Paitak, Jr., M., and Grill, L. 1993. Rapid, high level expression of biologically active alpha-trichosanthin in transfected plants by a novel RNA viral vector. Proc. Natl. Acad. Sci. USA 90:427-430.

Hilf, M. E., and Dawson, W. O. 1993. The tobamovirus capsid protein functions as a host-specific determinant of long-distance movement. Virology 193:106-114.

Chapman, S., Lindbeck, A. G. C., and Dawson. W. O. 1993. The structure of viral coat protein and its role in disease. Trends in Microbiol. 1:280-282.

Non-refereed publications:

Dawson, W. O., and Hilf, M. E. 1992. Host range determinants of plant viruses. Annu. Rev. Plant Physiol. and Plant Molec. Biol. 43: 527-555.

Dawson, W. O., and K. Lehto. 1990. Regulation of tobamovirus gene expression. Adv. Virus Res. 38: 307-342.

Dawson, W. O. 1990. Relationship of tobacco mosaic virus gene expression to movement within the plant. In "Viral Genes and Plant Pathogenesis", T. P. Pirone and J. G. Shaw, eds. pp. 39-52. Springer-Verlag, New York.

Culver, J. N., Lindbeck, A. G. C., and Dawson, W. O. 1991. Virus-host interactions: Induction of chlorotic and necrotic responses in plants by tobamoviruses. Annu. Rev. Phytopathol. 29:193-217.

Dawson, W. O. 1991. The pathogenicity of tobacco mosaic virus. Seminars in Virology 2: 131-137.